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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 08/940,692
Filing Date: September 30, 1997
Appellant(s): Valle et al.

Lynn Marcus-Wyner
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed August 27, 2003.

(1) Real Party in Interest

A statement identifying the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

A statement identifying the related appeals and interferences which will directly affect or be directly affected by or have a bearing on the decision in the pending appeal is contained in the brief.

(3) Status of Claims

The statement of the status of the claims contained in the brief is correct.

(4) Status of Amendments After Final

The amendment after final rejection filed on 8/27/03 has been entered.

(5) Summary of Invention

The summary of invention contained in the brief is correct.

(6) Issues

The appellant's statement of the issues in the brief is correct.

(7) Grouping of Claims

Appellant's brief includes a statement that the claims do not all stand or fall together but should be grouped in three separate groups A. Claims 23-26, B. Claims 38, 46, and 49, and C Claims 27, 29-31, 33-37, 42, 44, and 50 and provides reasons as set forth in 37 CFR 1.192(c)(7) and (c)(8).

(8) Claims Appealed

The copy of the appealed claims contained in the Appendix to the brief is correct.

(9) Prior Art of Record

The following is a listing of the prior art of record relied upon in the rejection of claims under appeal.

5,168,056	Frost	12/1/92
5,602,030	Ingrahm et al.	2/11/97

W. H. Holms, "The Central Metabolic Pathways of Escherichia coli: Relationship Between Flux and Control Branch Point, Efficiency of Conversion to Biomass, and Excretion of Acetate", Curr. Topics Cell. Regulation. 28: 69-105. (1986).

M.H. Saier et al. "Characterization of Constitutive Galactose Permease Mutants in *Salmonella typhimurium*", J. Bacteriol. 113(1): 512-514. (1991).

(10) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claims 23, 27, 38, 46, and 49 stand rejected under 35 U.S.C. 103(a) as being unpatentable over the combined disclosures of Saier et al and Ingrahm et al.

Saier et al. teach methods of selecting a Pts^-/$glucose^+$ *S. typhimurium* strain comprising deleting the PTS genes (*ptsH* and *ptsI*), culturing the mutant cell using glucose as the sole available carbon source and selecting cells with a fast growth rate on glucose. The mutant cells of Saier et al. use the galactose permease for the transport of glucose (page 512). The fastest growth rate specifically obtained by the mutants of Saier et al. was 0.35/hr. Applicants claimed methods recite selecting cells with a growth rate of at least 0.4/hr.

Ingrahm et al. teach that it would be advantageous to increase the supply of PEP in a cell used for production of a desired product, in particular aromatic amino acid production, by modifying an enteric bacteria such as *E. coli* to use an alternative pathway from the PTS system for glucose uptake such that PEP production is not obligately coupled to glucose transport (see column 3 lines 37-39).

Therefore, as Ingrahm et al. disclose that Pts$^-$/glucose$^+$ cells are particularly useful for production of desired products which use PEP as a precursor, one of ordinary skill in the art would have been motivated to screen for Pts$^-$/glucose$^+$ cells such as those of Saier et al. with even higher growth rates than those specifically disclosed by Saier et al. As Saier et al. disclose cells with growth rates very close to the claimed rate of at least 0.4/hr one of ordinary skill in the art would have reasonably expected to be able to obtain cells within the scope of the claims.

Claims 23-27, 29-31, 33-38, 42, 44, 46, 49, and 50 are rejected under 35 U.S.C. § 103 as being unpatentable over the combined disclosures of Frost, Holms, Ingrahm et al. and Saier et al.

Frost teaches the amplification of carbon flow into the common aromatic pathway by increasing the amount of one of the substrates (E4P) for the first committed step of this pathway (i.e., the DAHP synthetase catalyzed condensation of E4P and PEP) by introduction of a transketolase gene into the host cell (see column 6, line 64 - column 7, line 2). He further teaches the introduction of one or more of the genes of the common aromatic pathway in such cells to further increase the amount of the desired final product (see column 7, lines 2-10).

Holms teaches that PEP within *E. coli* is consumed by several different metabolic pathways (i.e., the PTS system, pyruvate synthesis by pyruvate kinase, and oxaloacetate synthesis by phosphoenolpyruvate carboxylase) and the amount of PEP channeled into each of these pathways. Holms teaches that the PTS system consumes 66% of the PEP produced while only 3% of the PEP pool is channeled into aromatic amino acid synthesis (see page 74).

Ingrahm et al. teach that it would be advantageous to increase the supply of PEP in a cell used for production of a desired product, in particular aromatic amino acid production, by modifying an enteric bacteria such as *E. coli* to use an alternative pathway from the PTS system for glucose uptake such that PEP production is not obligately coupled to glucose transport (see column 3 lines 37-39).

Saier et al. teach methods of selecting a Pts^-/$glucose^+$ *S. typhimurium* strain comprising deleting the PTS genes (*ptsH* and *ptsI*), culturing the mutant cell using glucose as the sole available carbon source and selecting cells with a fast growth rate on glucose. The mutant cells of Saier et al. use the galactose permease for the transport of glucose (page 512). The fastest growth rate specifically obtained by the mutants of Saier

et al. was 0.35/hr. Applicants claimed methods recite selecting cells with a growth rate of at least 0.4/hr.

The disclosure of Frost of amplification of carbon flow into the common aromatic pathway by increasing the amount of one of the substrates (E4P) for the first committed step of this pathway would suggest to the ordinary skilled artisan the amplification of the other necessary precursor (i.e., PEP) of this enzymatic step as this would assure that neither substrate for this enzyme would be in limiting supply. One of ordinary skill in the art would recognize that the supply of any precursor used by a cellular pathway could be amplified by either increasing the amount of the precursor synthesized (such as done by Frost for E4P) or by preventing the depletion of the precursor by other cellular pathways thereby increasing the amount of the precursor available to be used by the desired pathway. The disclosure of Holms that 66% of the cellular PEP is used by the competing PTS pathway would suggest to the ordinary skilled artisan that PEP availability to the common aromatic pathway could be substantially increased by preventing PEP use by the PTS pathway. Furthermore, Ingrahm et al. explicitly suggest this as an approach to increasing the level of carbon flow into the common aromatic pathway. The disclosure of Saier et al. shows that it is possible to produce cells which are deleted in the PTS system

yet still retain high growth rates on glucose (a carbon source normally transported by the deleted PTS system) by utilizing the galactose permease as a means of glucose transport. Therefore, it would have been obvious to one of ordinary skill in the art to produce a $Pts^-/glucose^+$ mutant of the host cells of Frost which exhibit high levels of carbon flow into the common aromatic pathway as one of ordinary skill in the art would reasonably expect such a mutant cell to divert higher levels of the cellular pool of PEP into the aromatic amino acid biosynthetic pathways and produce further increases in the amount of carbon flow into this pathway. It would have been further obvious to one of ordinary skill in the art to select for such cells with high growth rates as such cells would be expected to be most useful for producing large amounts of aromatic amino acids. As Saier et al. disclose $Pts^-/glucose^+$ cells with growth rates very close to the claimed rate of at least 0.4/hr one of ordinary skill in the art would have reasonably expected to be able to obtain cells within the scope of the claims. Furthermore, it would have been further obvious to one of ordinary skill in the art to further increase the amount of PEP diverted into this pathway by preventing its use by the other metabolic pathways which Holms teach that it is consumed by. As such it would have been obvious

to further mutate the pyruvate kinase and pyruvate carboxylase genes as well.

(11) Response to Argument

Appellants contend there is no suggestion or motivation provided by either the Saier et al. or the Ingrahm et al. references when taken alone or in combination which would suggest the mutant cells of Claim 23. Appellants further argue that the rejection of the cells of Claim 23 should be withdrawn as the combination of references lack any expectation of success in obtaining $Pts^-/glucose^+$ cells requiring galactose permease activity and having a specific growth rate on glucose as a sole carbon source of at last 0.4/hr.

Appellants argument that there is no suggestion or motivation provided by Saier et al. or Ingrahm et al. is clearly incorrect. Ingrahm et al. provide an **explicit** statement that providing a recombinant organism in which glucose uptake is not obligately coupled to PEP production is desirable. See column 3, lines 37-39. Furthermore, Ingrahm et al. state in column 3, lines 23-28 that by so modifying an enteric bacteria such as E. coli to use an alternative pathway for glucose uptake, the output of any synthetic product derived from a PEP precursor could be

doubled. As such motivation for the invention of Claim 23 is clearly present.

The majority of appellants argument for the patentability of Claim 23 over Saier et al. and Ingrahm et al. on pages 9-11 of the Appeal Brief argues that there is no expectation of success in obtaining a Pts^-/$glucose^+$ cells requiring galactose permease activity and having a specific growth rate on glucose as a sole carbon source of at last 0.4/hr. First it should be noted that Saier et al. report generation times using only one significant digit in the numbers reported such that a growth rate of 0.4/hr (which is equivalent to a generation time of 1.7 hrs using two significant figures) would have been reported as 2 hrs when rounded off to a single significant digit. The 2 hr generation time reported by Saier could correspond to any growth rate of from .28/hr (equivalent to a generation time of 2.4 hrs) up to .46/hr (equivalent to a generation time of 1.5 hrs) when two significant digits are considered. As such it is not even certain that the growth rate of the two mutant strains reported by Saier et al. do not in fact meet the limitations of the instant claims. However, even if the growth rate of these mutant strains isolated by Saier et al. is slightly lower than the 0.4/hr limitation recited in the claims (which was presumed in the rejection), it is certainly sufficiently close that one of

ordinary skill in the art would have expected to be able to isolate similar Pts$^-$/glucose$^+$ cells with growth rates as claimed. There is no evidence in Saier et al. or the prior art of major differences in cells with growth rate differences as small as 0.05/hr.

Appellants state that the presence of a GalR mutation such as is present in the mutants of Saier et al. when introduced into Pts$^-$ *E. coli* as reported on pages 15 and 16 of the specification only partially restored the ability of cells to utilize glucose. However, this observation is not on point to the central issue of whether there is a reasonable expectation of success in isolating Pts$^-$/glucose$^+$ cells with growth rates of at least 0.4/hr in view of the art showing of 2 Pts$^-$/glucose$^+$ cells with growth rates of 0.35/hr. There is no question that the cells of Saier et al. exhibit a glucose$^+$ phenotype. Therefore, the failure of the appellants to produce a glucose$^+$ phenotype by introducing a GalR mutation is irrelevant. The cells of Saier et al. are clearly phenotypically different from the GalR mutants discussed on pages 15 and 16 of the specification and were clearly produced by a different process which was designed to eliminate all cells with a glucose$^-$ phenotype. The cells of Saier et al. may include other mutations in addition of the GalR mutation or may simply

have different GalR mutations which produce different phenotypic effects. Merely because some GalR mutants have a glucose$^-$ phenotype as shown by appellants does not lead to a conclusion that one could not use the disclosure of Saier et al. to produce Pts$^-$/glucose$^+$ cells similar to those of Saier et al. What is important is the showing by Saier et al. that growing Pts$^-$ cells on a medium with glucose as the sole carbon source results in Pts$^-$/glucose$^+$ cells which include a mutation in GalR that allows transport of glucose and growth rates very similar to those claimed by appellants.

Appellants also point out that the alternative pathway for glucose transport taught by Ingrahm et al. is distinct from the pathway recited in applicants claims and state that at the time of the instant invention several reports indicated that under glucose limited conditions the MglABC transport system was the preferred system for glucose transport. However, the rejection has never maintained that the alternative pathway for glucose transport taught by Ingrahm et al. is identical to that claimed. Ingrahm et al was cited solely to show **motivation** to look for additional mutant cells as taught by Saier et al. with growth rates as recited in the instant claims. The fact that other pathways are available for restoring glucose transport to Pts$^-$

cells such as that taught by Ingrahm et al. or that of Death et al. (cited by appellants) does not indicate that one of skill in the art would not reasonably expect to be able to isolate Pts$^-$/glucose$^+$ cells as taught by Saier et al. with growth rates of at least 0.4/hr. If anything the fact that the art was aware of other alternative pathways for providing glucose transport in addition to mutation of GalR would raise the expectation that one could obtain Pts$^-$/glucose$^+$ cells with slightly higher growth rates than those obtained by Saier et al. as one would expect that additional mutations in the regulatory genes of these other pathways might further increase the ability of the Pts$^-$/glucose$^+$ cells of Saier et al. to restore glucose transport. It should be noted that the Death et al. reference cited by appellants to support this argument, states that PTS enzyme II mutants of *Salmonella typhimurium* (i.e., Pts$^-$ cells) in glucose-limited chemostats accumulated *mgl*-constitutive mutations. Thus under selective conditions clearly there are multiple different mutations which can be produced which provide restoration of glucose transport in the absence of the PTS system. As such one would clearly expect that one could obtain Pts$^-$/glucose$^+$ cells with slightly higher growth rates than those obtained by Saier et al.

Arguing for the patentability of Claims 38, 46, and 49 which are all drawn to methods of isolating the mutant Pts$^-$/glucose$^+$ cells appellants state that they incorporate all arguments presented for patentability of Claim 23 and further contend that exposing cells to a mutagenizing agent such as nitrosoguanidine and then selecting mutants from minimal glucose agar medium is different from a method of using a continuous culture, such as a chemostat, in order to select glucose$^+$ revertants of the PTS$^-$ strains having at least a specific growth rate of 0.4/hr on glucose. The examiner also explicitly incorporates all responses to the arguments presented with regard to Claim 23 herein with regard to Claims 38, 46, and 49. Furthermore, the examiner has never contended that the methods of Saier et al. of exposing cells to a mutagenizing agent such as nitrosoguanidine and then selecting mutants from minimal glucose agar medium is identical to the method of using a continuous culture with glucose as a carbon source in order to select glucose$^+$ revertants as recited in these claims. Instead as previously stated both methods would have been recognized in the art as equivalent alternatives for isolating mutants of Pts$^-$ cells that would grow on glucose as the sole carbon source. The method by which the mutation is generated (whether chemically induced or spontaneous) is irrelevant to a showing that such mutations can be isolated.

Applicants have not provided any reason why the two methods would not be considered equivalent. The only statement by appellants remotely resembling a **reason** why the two methods would not be considered equivalent is found on page 13 of the Brief where appellants state that instead of using a method capable of causing multiple mutations in the same strain as disclosed by Saier, appellants' purposely choose a continuous culture method which would exploit the natural capability of the cells to generate spontaneous mutations under stressful conditions and would eliminate mutant strains with a growth rate lower than 0.4 hr^{-1}. However, this is not persuasive because the there is no reason to believe that multiple mutations would be detrimental in the method of Saier et al. as the method of Saier et al. includes selective pressure to eliminate detrimental mutations as well. **Both** methods include selective pressure for those mutants that can successfully transport glucose and selective pressure against those cells which cannot transport glucose or against those cells with other mutations detrimental to growth.

Appellants argue for the separate patentability of Claim 27 the other claims rejected however section 8.B.1(c) which purportedly contains reasons for the separate patentability of this claims merely reiterates the conclusions previously stated

for the other claims rejected over the combination of Saier et al. and Ingrahm et al. Claim 27 recites a method for increasing PEP availability to a biosynthetic or metabolic pathway of a host cell, the method comprising, selecting a cell Pts$^-$/glucose$^+$ having the limitations of the cells of Claim 23 and culturing these cells. As Ingrahm et al. **explicitly** state that providing a recombinant organism in which glucose uptake is not obligately coupled to PEP production is desirable and that by so modifying an enteric bacteria such as E. coli to use an alternative pathway for glucose uptake, the output of any synthetic product derived from a PEP precursor could be doubled, it is unclear how the method of Claim 27 could be considered nonobvious if the cells (i.e., Claim 23) are obvious as explained above as the art clearly teaches what one would wish to use such cells for. As such the two claims should stand or fall together. Either both the cells and the method are obvious for all the reasons expressly stated by the examiner above, or both the cells and the method are non-obvious.

Arguing for the patentability of Claims 23-27, 29-31, 33-38, 42, 44, 46, 49, and 50 over the combined disclosures of Frost, Holms, Ingrahm et al. and Saier et al. appellants state that the addition of Frost and Holms appears to be provided to address the

limitations of (i) recombinant DNA encoding enzymes such as transketolase, transaldolase, and pyruvate synthase or (ii) mutations in genes encoding pyruvate kinase and they incorporate all arguments presented for patentability of Claims 23, 27, 38, 46, and 49. The examiner acknowledges that this these additional references were primarily added to address the additional limitations as discussed by appellants. However, the examiner believes that the additional references further bolster the argument for obviousness presented above even for those claims which do not recite these limitations. As such Claims 23, 27, 38, 46, and 49 were included in the rejection over Frost, Holms, Ingrahm et al. and Saier et al. as well as in the rejection previously discussed. All previous arguments discussed in the rejection of Claims 23, 27, 38, 46, and 49 over Ingrahm et al. and Saier et al. are expressly incorporated herein with regard to the rejection of Claims 23-27, 29-31, 33-38, 42, 44, 46, 49, and 50 over the combined disclosures of Frost, Holms, Ingrahm et al. and Saier et al. Applicants discussion on pages 15-17 does not appear to add **any** new arguments to those already presented for the rejections of Claims 23, 27, 38, 46, and 49 over Ingrahm et al. and Saier et al. except to piecemeal elaborate on the elements that are missing from each of the references

individually with regard to the instant claims and to argue that Frost and Holms do not remedy the deficiencies found in the combination of Ingrahm et al. and Saier et al. As the rejection was made under 35 U.S.C. § 103 it has never been the contention of the examiner that any one of the cited references taken alone taught or made obvious the claimed invention. However, it is the combined disclosures that clearly make obvious the invention. One cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

For the above reasons, it is believed that the rejections should b sustained.

Respectfully submitted,

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December 23, 2003

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